

## REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and the following commentary.

### **I. Introduction**

Claim 19 is cancelled without prejudice or disclaimer. Applicant reserves the right to pursue the subject matter of any cancelled claim in a continuing application.

Claim 8 is amended to state that the minicells are loaded with a therapeutically significant concentration of a small molecule drug. Support for the amendment is found in the originally filed specification. For instance, page 10, lines 21-23 provides that an “unexpected aspect of the present invention is that therapeutically significant drug concentrations can be packaged within minicells.”

Claims 31-36 are new claims with support found in the originally filed specification. In particular, the specification provides a minicell composition containing a drug (page 12, lines 1-5) and states that the drug can be a small molecule drug (page 13, lines 3-7) or a cancer chemotherapy drug (page 12, line 5). The specification further provides that the small molecule drug can be loaded into the minicells to reach a therapeutically significant concentration (page 10, lines 21-23). Moreover, the minicell composition can contain fewer than about 1 contaminating parent bacterial cell per  $10^{10}$  minicells or per  $10^{11}$  minicells (*id.* at page 14, lines 10-12). Finally, bispecific ligands and their capabilities to activate receptor-mediated are described on page 15, line 27 to page 16, line 5 and page 16 lines 23-26.

Because no new matter is introduced, entry of the amended claim 8 and the new claims 31-36 is respectfully requested.

Applicant further submits that the new claims are directed to the elected invention. In the Requirement of Restriction mailed October 6, 2008, Examiner Singh divided the originally filed claims into three invention groups: Group I (claims 1-7 and 29, directed to compositions of minicells); Group II (claims 8-27 and 30, directed to drug delivery methods; and Group III (claims 28, directed to methods of loading minicells with a drug). According to the examiner, “Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features” in view of Sabbadini et al. (US Patent 7,183,105). Requirement for Restriction at page 3, lines 3-8. The examiner alleges that Sabbadini teaches “an intact minicell that contains a functional nucleic acid” (*id.* at page 3, line 9) and the

content of the minicell is delivered into a cell (*id.* at page 3, line 18). In response, applicant elected Group II (claims 8-27 and 30) for examination.

New claims 31-36, albeit composition claims, relate to the same single general inventive concept as the claims of group II because both independent claims 31 and 8 recite a minicell that is “loaded with a therapeutically significant concentration of a small molecule drug.” As the specification states, the inventors discovered unexpectedly that “therapeutically significant drug concentrations can be packaged within minicells” (page 10, lines 21-23). As neither Sabbadini nor any other cited art discloses a minicell loaded with a therapeutically significant concentration of a small molecule drug, such a minicell constitutes a common general inventive concept. Accordingly, the new claims relate to the same inventive concept, under PCT Rule 13.2, as the claims of Group II. Joinder of the new claims to the elected invention Group II, therefore, is respectfully requested.

Upon entry of the amendment and entry and joinder of the new claims, claims 8, 10-15, 17, 18, 20-22 and 31-36 will be pending.

## **II. Rejection Under § 102**

Claims 8, 10-1, 15, 17-19, 21 and 22 stand rejected for alleged anticipation over Sabbadini *et al.* Claim 19 has been cancelled. Applicant traverses the rejection of the remaining claims.

Amended claim 8 is directed to a targeted drug delivery method using a composition that includes intact, bacterially derived minicells that are *loaded with a therapeutically significant concentration* of a small molecule drug. The composition further includes bispecific ligands having specificity for a mammalian cell surface receptor and specificity for said minicells. By contacting the composition with non-phagocytic mammalian cells, (i) the bispecific ligands cause the minicells to bind to the mammalian cells, (ii) the minicells are engulfed by the mammalian cells, and (iii) the small molecule drug is released into the cytoplasm of the mammalian cells.

### **1. Sabbadini does not teach a minicell loaded with a therapeutically significant concentration of a small molecule drug**

According to Examiner Singh, Sabbadini teaches “a targeted drug delivery method comprising contacting a target non-phagocytic mammalian cell with an intact bacterially derived minicell coated with an antibody as a binding moiety capable of binding to a ligand present on the surface of the target mammalian cell, wherein the minicell comprises the small molecule, and

wherein the contents of the minicell are delivered into the cell from a minicell bound to the cell.  
Pending Action at page 2, line 16 to page 3, line 2.

Even as interpreted by the examiner, however, Sabbadini does not teach that the minicells are *loaded with a therapeutically significant concentration* of a small molecule drug. In fact, as the specification provides, it was the inventors' discovery that minicells can be loaded with "therapeutically significant concentrations" of small molecule drugs (specification at page 10, lines 21-23) and the drugs do not "move out of the minicells" after the loading (*id.* at page 24, lines 22 and 23). Sabbadini, by contrast, does not disclose a minicell loaded with a therapeutically significant concentration of a small drug.

For this reason alone, Sabbadini must fail a Section 102 reference.

**2. Sabbadini's minicells are different from the minicells of the claimed invention**

As defined in the specification, "minicell" refers to "anucleate forms of *E. coli* or other bacterial cells, engendered by a disturbance in the coordination, during binary fission, of cell division with DNA segregation" (page 12, lines 6-8). By contrast, Sabbadini defines "minicell" to encompass all derivatives of cells that lack chromosomal DNA, *i.e.*, all achromosomal cell derivatives (see column 38, lines 52-58). The minicells of Sabbadini, therefore, convey a concept or category of "minicell" that is different from the intact, bacterially-derived minicells of the claimed invention.

First, the minicells of the claimed invention do not embrace all manner of cell derivative that lacks a nucleus. For instance, it is known that membrane blebs are anucleate vesicles that, by spontaneous blebbing of bacterial membrane, form in the course of normal bacterial growth. These membrane blebs fall into the definition of Sabbadini's minicell as they lack chromosomal DNA. But these membrane blebs are not minicells within the meaning of the instant application because they do not result from *cell division*. Accordingly, these "membrane blebs" are removed from minicell preparation by way of filtration (see specification at page 15, lines 18-21).

The differences between Sabbadini's minicells and those of the claimed invention are also apparent from the differences of their sizes. Sabbadini's "minicell" can have any size from about 0.005, 0.1, 0.15, 0.2 to about 0.25, 0.3, 0.35, 0.4, 0.45 or 0.5 micrometers (column 38, lines 30 and 31). By contrast, the minicells of the claimed invention are free of any particle, including membrane

blebs, that are 0.2 micrometers or smaller (see specification at page 15, lines 18-21). Clearly, Sabbadini does not disclose the minicell of the claimed invention.

In response to this argument, the examiner argued that “the feature upon which applicant relies (i.e., size of minicell diameter” are not recited in the rejected claims” (pending action at page 6, last paragraph). Applicant notes that the size of minicells of the claimed invention is an *inherent* feature of the minicells as defined in the specification. The comparison of the sizes of the Sabbadini’s minicells and the minicells of the claimed invention is proffered to *demonstrate the differences* on the concept, rather than further limit the claimed invention.

From the foregoing, it is apparent that Sabbadini does not teach every element of the claimed invention. Withdrawal of the Section 102 rejection is warranted, therefore.

### **III. Rejections Under § 103**

The examiner rejects claims 8, 11, 13-14 for alleged obviousness over Sabbadini *et al.*, Nettelbeck *et al.* and Colwell *et al.* Claims 8 and 20 stand rejected separately over Sabbadini *et al.* and Hope *et al.* Applicant traverses the rejections.

The claimed invention is described above. The examiner’s interpretation of Sabbadini is also briefly described above. According to the examiner, Nettelbeck teaches “a recombinant antibody as a molecular bridge” (pending action at page 8, lines 24-25) and Colwell teaches “production of monoclonal antibodies to antigenic determinants of the O-polysaccharide of ... LPS” (*id.* at page 9, lines 1-3).

The examiner further alleges that, “prior to instant invention, it was routine in the art to package/load chemotherapeutic drugs such that diffusion across the phospholipid bialyer-membrane is unidirectional or targeted delivery of the molecule” (*id.* page 10, lines 3-5). To support this allegation, the examiner relies on Hope for teaching a method that “involves loading a chemotherapeutic agent such as doxorubicin into preformed lipid bilayers of liposome having a concentration gradient across the lipid bilayers (*id.* at page 10, lines 5-7). The examiner further alleges that “the structure of the lipid bilayers is similar to the membranes enveloping animal cells” (*id.* at page 10, lines 8-9). The examiner then goes on to conclude that it would have been obvious to the skilled artisan to load “chemotherapeutic agent such as doxorubicin into minicell using the method disclosed by Hope” (*id.* at page 10, lines 10-13).

As explained above, however, Sabbadini does not disclose the intact, bacterially derived minicells of the claimed invention. Nor does any secondary reference teach a minicell that is an “anucleate form[] of *E. coli* or other bacterial cells, engendered by a disturbance in the coordination, during binary fission, of cell division with DNA segregation,” as the claimed invention prescribes.

For this reason alone, a *prima facie* case of obviousness is not established and the Section 103 rejection must fail. Moreover, the examiner’s argument that analogizes minicells to liposomes is misplaced.

**1. Hope does not teach or suggest that a small molecule drug can be loaded into a minicell by a concentration gradient**

The examiner alleges that, for liposomes, “the structure of the lipid bilayers is similar to the membranes enveloping animal cells” (*id.* at page 10, lines 8-9) and thus it would have been obvious to load “chemotherapeutic agent such as doxorubicin into minicell using the method disclosed by Hope” (*id.*, lines 10-13). This argument is misplaced.

***A. Liposome bilayers are not similar to cell membranes***

First, Hope does not analogize a liposome bilayers to a cell membrane, as the examiner alleges.

The structure of the lipid bilayers is similar to the membranes enveloping animal cells, and are a result of amphipathic lipids arranged such that the hydrophobic portions of the lipid orient toward the center of the bilayers while the hydrophilic headgroups orient towards the inner or outer aqueous phases.

Page 1, lines 20-24. Thus, Hope does not teach that lipid bilayers of liposomes are similar in general to cell membranes. Rather, as evidenced by the connective phrase “such that,” Hope invokes the comparison in order to illustrate the cell membrane-like *orientations* of liposome lipids.

Second, the similarity between liposome bilayers and animal cell membranes, with respect to the orientation of constituent lipids, does not suggest that, like the liposome bilayers, animal cell membranes are permeable to a chemotherapeutic agent. This is not even true with bacterial cell membranes, which are simpler than animal cell membranes. For instance, the specification states that “live bacterial cells exhibit selective membrane permeability to solutes” and “extrude noxious chemicals that enter into the bacterial cytoplasm” (page 10, lines 12-17). Further, “bacterial cytoplasm ... contains significant concentrations of biocompatible solutes” and “it was believed that

there might be insufficient spare intracellular space to accommodate high concentrations of non-biocompatible drug solutes” (*id.* at page 10, lines 24-26).

Third, Hope’s methodology for introducing a chemotherapy drug into a liposome clearly is not properly generalized to intact, bacterially derived-minicells. The prior-art methodology is illustrated in Hope’s Figure 1 (see also claim 1) and is explained in the specification:

[L]iposomes are formed having an encapsulated medium which contains a methylammonium salt. The external medium which originally has the composition of the encapsulated medium is exchanged with a neutral external medium. A therapeutic agent such as doxorubicin or ciprofloxacin (structures shown in Figure 2) which is both lipophilic and which can be protonated is drawn toward the liposomes’ encapsulated medium by both its polarity and the methylammonium ion gradient which is established across the bilayer. Methylamine diffuses out of the liposomes as the therapeutic agents are drawn in and protonated, thereby maintaining the differential (see Figure 1).

Page 3, lines 11-19 (emphasis added).

It is apparent that features of (i) the *methylammonium salt* encapsulated in the liposome, (ii) the *methylammonium ion gradient*, which is established across the bilayer by virtue of removal of encapsulated medium, and (iii) the subsequent *diffusion of methylamine* out of the liposome are all critical for the therapeutic agent to be drawn into the liposome. This method is only applicable to liposomes, which can be *formed* in a medium containing a methylammonium salt. By contrast, a minicell cannot be thus formed but rather is produced intact during cell division. The skilled artisan would have readily appreciated, therefore, that the methodology disclosed in Hope would not be successful in loading a small molecule drug into minicells, per the claimed invention.

In summary, there is a substantial, art-recognized differences separating liposomes from intact, bacterially-derived minicells, as recited. Further, the Hope method clearly is inapplicable to such minicells. Given these considerations, an asserted similarity between liposomes and cells in relation to membrane structure, vis-à-vis the orientation of constituent lipids, would not have suggested that the Hope methodology somehow might work for intact, bacterially-derived minicells.

***B. The present invention achieves unexpected results***

The specification also amply documents reasons why the ability of intact, bacterially-derived minicells to package drug would have been unexpected.

First, it was “surprising that that intact minicell membranes are permeable to a range of structurally dissimilar hydrophilic, hydrophobic and amphipatic drugs” in view of the conventional wisdom that “live bacterial cells exhibit selective membrane permeability to solutes” (specification at page 10, lines 12-14).

Second, it was “surprising that minicells are unable to expel drugs from their cytoplasm, because live bacterial cells extrude noxious chemicals that enter into the bacterial cytoplasm. Even against a reverse osmotic gradient, in which drug- loaded minicells are suspended in phosphate-buffered saline containing no drug,” intact, bacterially-derived “minicells retain drug. This is additionally surprising because drugs appear simply to diffuse into minicells through intact minicell membranes, yet the diffusion channels are *not available for drugs to diffuse out of minicells.*” *Id.* at page 10, lines 15-21 (emphasis added).

“Another unexpected aspect of the present invention is that *therapeutically significant drug concentrations* can be packaged within minicells, because bacterial cytoplasm (and, hence, minicell cytoplasm) contains significant concentrations of biocompatible solutes. Thus, it was believed that there might be insufficient spare intracellular space to accommodate high concentrations of non-biocompatible drug solutes, without loss of minicell integrity.” *Id.* at page 10, lines 21-27 (emphasis added).

Fourth, the ability of minicells to deliver drugs effectively also is surprising for various reasons described in the specification at page 10, line 28 to page 11, line 26. For example, it was unexpected that drug-packaged minicells do not leak drug into extracellular space (*id.* at page 10, line 29-30) and the drug-packaged minicell can avoid degradation. *Id.* at page 11, lines 17 and 18.

It is not surprising, therefore, that the inventors’ findings were published as a well-received article in the prestigious journal, *Cancer Cell*. See MacDiarmid *et al.*, *Cancer Cell* 11: 431-45 (2007), submitted concurrently with a supplemental information disclosure statement. The surprising character of the published results, as with the presently claimed invention, prompted an editorial in *Nature Reviews Drug Discovery*, which features that invention. Flemming, *Nature Reviews Drug Discovery* 6: 519 (2007), also submitted concurrently, thus concluded that “these results might form the basis of a robust and versatile drug carrier system.” Likewise highlighting the claimed invention, Geddes in *The New Scientist*, 12 May 2007 at page 8 (copy submitted), noted that a cancer research scientist at Beth Israel Deaconess Medical Centre (Boston, MA) was “impressed” by the inventors’ minicell-based approach.

In the face of all of these points favoring patentability, the examiner contends that they are unpersuasive because the “claims are not limited to therapeutically significant concentrations of drug” (pending action at page 11, lines 6 and 7). Without acquiescing to this contention, applicant notes that the examiner’s stated concern is inapposite to the present claims, which recite that the minicells are loaded with a *therapeutically significant concentration* of a small molecule drug (see claim 8).

Further, the examiner alleges that “the arguments of counsel cannot take the place of evidence in the record ... Applicants have not provided an appropriate affidavit or declaration supporting that the specific limitations and conditions as set forth in argument is *effective* in delivering the drug to a target cell[.]” *Id.* at page 11, lines 11-17; original emphasis).

The points recounted above are not mere “attorneys argument,” however. In relation to an alleged *prima facie* case, as here, “[r]ebuttal evidence and arguments can be presented in the specification ... *or* by way of an affidavit or declaration under 37 CFR 1.132” (MPEP § 2145; emphasis added), and the PTO is obliged in either instance to weigh the proffered evidence. Thus, “Office personnel should consider all rebuttal arguments and evidence presented by applicants.” See, *e.g.*, *Soni*, 54 F.3d at 750, 34 USPQ2d at 1687 (**error not to consider evidence presented in the specification**).” *Id.* (emphasis added).

It is error, therefore, to discount the “evidence and arguments” presented in applicant’s specification simply because that material is not in declaration form. Nevertheless, without acquiescing to the examiner’s rationale, applicant has filed concurrently a declaration by the inventors.

In view of the foregoing, applicant submits that the Section 103 rejection is without merit and should be withdrawn.

### **III. Double-Patenting Rejections**

The examiner provisionally rejects claims 8, 16, 19-22 under obviousness-type double patenting over claims 40-44, 48-51 and 73 of U.S. application No. 11/765,635. Applicant understands the examiner to mean claim 17 in this regard, since claim 16 was cancelled in the previous response. Claim 19 is requested to be cancelled now, too, rendering the rejection moot. Applicant respectfully traverses the rejection of the remaining claims.



First, the '635 application is directed to compositions and methods relating to killed bacterial cells. Killed bacterial cells are non-living bacterial cells (published '615 application at paragraph 0047). By contrast, the minicells of the claimed inventions are engendered by a disturbance in the coordination, during binary fission, of cell division with DNA segregation. Thus, killed bacterial cells are different from and not suggestive of intact, bacterially-derived minicells.

In addition, claims 40-44, 48-51 and 73 have been cancelled from the '635 application via an amendment filed on January 5, 2010, rendering the rejection moot. Withdrawal of the double-patenting rejection, therefore, is respectfully requested.

**IV. Information Disclosure Statement**

Applicant's representative received a letter, dated February 26, 2010, from Ryan E. Melnick under the letterhead of Knobbe Martens Olson & Bear LLP, the agent named on U.S. Patent No. 7,183,105 to Sabbadini *et al.*, presently cited. Accompanying the letter were nine publications that, Mr. Melnick alleged were material to the subject application. The letter and the publications are submitted concurrently with the supplemental information disclosure statement.

Applicant provides the following in response to the Melnick letter. The last part of the letter addresses commonly assigned, co-pending application serial No. 11/211,098. As these statements relate as well to the aforementioned publications, they are addressed here as well.

**1. Hale *et al.* does not describe that minicells entered HeLa cells via receptor-mediated endocytosis**

The first part of the Melnick letter relates to the subject application and a co-pending application serial No. 10/581,990. In the paragraph bridging pages 1 and 2, Mr. Melnick stated:

[y]ou have argued that skilled artisans would have no reason to expect that particles as large as intact bacterially derived minicells of at least 400nm in diameter could be readily taken up by non-phagocytic mammalian cells via receptor-mediated endocytosis. However, Hale *et al.* (1983) discloses that bacterial minicells with a size range of about 400 nm in diameter are endocytosed into HeLa cells (a non-phagocytic human cervical cancer cell line) when proteins are present on the surface of minicells that are capable of stimulating receptor-mediated endocytosis. Specifically, Figures 4A and 4B (Hale 1983) depict minicells from *Shigella* that contain specific endocytosis-inducing outer membrane proteins encoded on and produced from the virulence plasmid of *Shigella flexneri* serotype 5 being repeatedly trafficked into HeLa cells (see description on page 346, column 1).

(Emphasis added.)

This statement is incorrect. Nowhere does Hale *et al.* describe a receptor-mediated endocytosis. Nor does the Hale publication mention a specific, endocytosis-inducing outer membrane protein, as Mr. Melnick alleges.

By way of introduction, the present inventors made the unheralded discovery that intact, bacterially derived minicells can be engulfed by a non-phagocytic mammalian cell via a receptor-mediated endocytosis process. Before this surprising discovery, it was well recognized in the art that large particles like minicells enter a non-phagocytic mammalian cell through an active and virulent invasive process. Such an active invasion may involve major molecular invasion proteins such as internalinA (InlA) and internalinB (InlB).

By contrast, receptor-mediated endocytosis is a passive and avirulent process. Exemplary such receptor-mediated endocytosis are clathrin-mediated endocytosis and caveolin-mediated endocytosis. See, generally, Doherty and McMahon, *Annu. Rev. Biochem.* 78: 31.1 – 31.46 (2009) (submitted concurrently with the supplemental information disclosure statement).

At the outset, Hale *et al.* states clearly that the goal of the study was to characterize the role of plasmids in their contribution to the virulent phenotype of natural bacteria, motivated by the observed association of plasmids' presence in bacteria and the bacteria's virulent phenotype. (see page 340 in column 2, lines 1-7). In this context, Hale *et al.* indicates that the subject of the study was "the association of extrachromosomal elements with virulence in invasive enteric pathogens." *Id.* at page 340, column 2, lines 8-10 (emphasis added). Anucleate minicells, which contained such extrachromosomal elements, *e.g.*, plasmids, were used for this purpose. *Id.* at page 340 in column 2, lines 17-20.

Apparently, Hale's "minicells" were useful because they did not contain chromosomes so that the invasive enteric virulent phenotype could only be attributed to the plasmids. Thus, the process for the plasmid-containing minicells to enter host cells was expected to be the same as that for natural bacteria, *i.e.*, active and virulent invasion.

Not surprisingly, Hale *et al.* reports that these minicells entered HeLa cells through an active and virulent invasive process, just like how a natural bacterium enters a non-phagocytic mammalian cell. Nowhere in the entire article does Hale *et al.* state or hint that the minicells entered the HeLa

cells through a different mechanism, or in particular, the receptor-mediated endocytosis process, as Mr. Melnick claimed it did.

The section heading on the bottom of page 345, second column, and the immediately following paragraph reflect the understanding of Hale and his co-authors about this process:

**Invasion of HeLa cells by *Shigella* minicells.** The study of plasmid-encoded virulence determinants in purified minicells was based on the assumption that these anucleate bacterial cells retain the virulence phenotype of vegetative parental cells ... Figure 4B shows a higher magnification of the process of minicell invasion ... Figure 4C shows minicells ... which had lost the ... plasmid did not establish intimate

contact with the plasma membrane. This behavior is consistent with the avirulent phenotype of the vegetative cells of this strain.

*Id.*, paragraph bridging pages 345 ad 346 (emphasis added).

Thus, Hale *et al.* confirms the author's perception that the process through which the plasmid-containing minicells entered HeLa cells was the same invasive and virulent process by which the parental cells accessed host cells. The publication also makes it clear that that plasmid-less minicells could not initiate such a process. To the contrary, the present inventors were the first to demonstrate that plasmid-less minicells can enter such mammalian cells, through a receptor-mediated endocytosis process. Thus, it is clear that the process observed in Hale *et al.* is different from receptor-mediated endocytosis, contrary to Mr. Melnick's statement.

In reference to Hale *et al.*, Mr. Melnick repeatedly employs the phrase "receptor-mediated." Yet, Hale *et al.* neither includes that phrase nor advances a similar concept. Not surprisingly, Mr. Melnick failed to pinpoint to any specific text in Hale *et al.* to substantiate his statements in this regard.

Mr. Melnick also relied on Pal *et al.* and Watarai *et al.* to support the proposition that the invasive process observed in Hale *et al.* is receptor-mediated endocytosis, induced by the outer membrane protein encoded by the plasmids. Yet, both Pal *et al.* and Watarai *et al.* describe bacterial invasion, a process wholly distinct from receptor-mediated endocytosis. For example, see Pal *et al.* at page 2580, first column, line 1, and Watarai *et al.*, page 991 (summary), line 6. Further, Pal *et al.* and Watarai *et al.* merely describe how plasmid-containing bacteria adhered to the mammalian cells

through Ipa invasin-integrin binding. Unlike clathrin and caveolin, binding of an invasin to integrin triggers virulent invasion rather than receptor-mediated endocytosis. It is apparent, therefore, that the processes disclosed in Pal *et al.* and Watarai *et al.* are not receptor-mediated endocytosis.

There is an extensive body of prior research papers and review articles that illuminates the active and virulent invasive process, through which bacteria attack mammalian cells. Hale *et al.* does not distinguish itself from this literature at all. Therefore, Mr. Melnick's reliance on Hale *et al.* to show minicell-induced, receptor-mediated endocytosis is simply a misreading of the prior literature.

**2. Hale et al. and Jaffe et al. do not show that minicells are about 400nm in diameter**

With reference to the present inventors' status as the first to determine that intact, bacterially derived minicells generally have a diameter of approximately 400nm, Mr. Melnick argued that:

...[t]he scale reference bar in the Figures 4A and 4B (Hale 1983) micrographs clearly indicates that the minicells are about 400nm in diameter. In addition, Jaffe *et al.* (1988) teaches that *E. coli* minicells are about 400nm in diameter (see Figure 2) as do many other references ...

(Page 2, second paragraph.) So stating, Mr. Melnick then asserted that applicant's submission concerning the inventors' discovery was wrong. Again, this assertion is itself erroneous.

First, Jaffe *et al.* in Figure 2 shows a size distribution graph of minicells. The sizes, based on the graph, range from about 250 nm to about 1500 nm. Jaffe *et al.* states that:

... [t]he size distribution of anucleate cells measured after DAPI staining, including spherical and rod-shaped anucleate cells, was broad than expected, although no anucleate filaments were detected (Fig. 2).

Page 3096, second column, lines 3-6, emphasis added. Again,

...[w]e observed that the *min* mutants spontaneously produced two types of anucleate cells, spherical minicells and anucleate rods. Both types of anucleate cells had a broad size distribution (Fig. 2). This was particularly surprising in the case of minicells, since cell diameter is constant in our experimental conditions.

*Id.* at page 3099, first column, lines 5-10, emphasis added.

It cannot be clearer, therefore, that Jaffe *et al.* actually discloses that the "minicells" under discussion had a broad range of sizes, in contravention of Mr. Melnick's statement.

With respect to the relevant figures in Hale *et al.*, applicant notes that Figure 4A, while lacking a reference scale bar *per se*, is clear in its indication that the “minicells” under discussion were characterized by a broad size range. This fact is not contradicted by Figure 4C, which Mr. Melnick fails to mention. Thus, Figure 4B does show three “minicells” that are about 400nm in diameter, but this does not negate the teaching of other figures, which show wide ranging “minicell” sizes. Furthermore, nothing in any of the Hale figures could have suggested that intact, bacterially derived minicells actually are uniformly sized, as the present inventor’s discovered.

Again, Mr. Melnick is incorrect in his statement about what the art understood about the size of minicell.

3. **The art expected that tumor cells would not repeatedly engulf minicells through receptor-mediated endocytosis**

The last part of the Melnick letter relates to the co-pending application serial No. 11/211,098. According to Mr. Melnick, Hale *et al.* demonstrated that tumor cells, *e.g.*, HeLa cells, endocytosed minicells repeatedly. Relying on Mamot *et al.* (2003), Dmitriev *et al.* (2000), and Desai *et al.* 1997, Mr. Melnick also stated that it was known in the art that tumor cells were capable of receptor-mediated endocytosis. Thus, the Melnick letter questioned applicant’s position on the unexpectedness that tumor cells can repeatedly engulf minicells through receptor-mediated endocytosis, as the inventors discovered. Again, Mr. Melnick’s arguments are meritless.

Applicant has explained above that Hale *et al.* does not describe a receptor-mediated endocytosis process. Mr. Melnick likewise misinterpreted the other publications cited in his letter.

By way of example, Desai *et al.* show that particles ranging in the size from 0.1µm to 10µm were “engulfed” by cancer cells. Applicant notes that these particles were introduced at high concentrations (100µg/ml) into the tissue culture medium. At such a high concentration, it is well-known that large particles can be taken up by cells through macropinocytosis, a clathrin-independent process. Further, even the authors’ own description contradicts Mr. Melnick’s statement.

The smaller diameter microparticles seems to have efficient interfacial interaction with the cell membrane compared to larger diameter microparticles. Probably the larger diameter microparticles (>1µm) are taken up by mechanism other than endocytosis, such as fluid-phase pericytosis and should be the subject for further investigation.

Desai *et al.* at page 1571, in the second column, “Discussion” (emphasis added).

It is beyond peradventure, therefore, that Desai *et al.* does not describe receptor-mediated endocytosis by tumor cells at all. Accordingly, Mr. Melnick’s statement is incorrect.

The art recognizes that tumor cells, like all other non-phagocytic mammalian cells, are incapable of taking up particles as large as intact, bacterially derived minicells through receptor-mediated endocytosis. It also was known that tumor cells, in particular, are resistant to repeated uptake of such large particles. Further, as explained in greater detail in the main response, the art was well-aware that tumor cells exhibit considerable genetic heterogeneity and are subject to frequent mutational change.

Chauhan *et al.*, *Br. J. Cancer* 88: 1327-34 (2003) and Pescharda and Park, *Cancer Cell* 3: 519-23 (2003), submitted concurrently with the supplemental information disclosure statement, illustrate the understanding in the art that tumor cells can mutate under selection so as to alter their capacity for endocytosis and/or lysosome function. By the same token, the skilled artisan would have expected that cancer cells would mutate a pathway, such as a receptor-mediated endocytosis pathway, that has the potential to kill the cancer cells.

Thus, it would have been expected that a cancer cell, after endocytosing a first minicell with an anti-oncology agent, would have been more likely to mutate the receptor-mediated endocytosis pathway to prevent uptake of a second minicell via the same process. This expectation further underscores the surprising nature of the present inventors’ discovery that tumor cells actually are capable of repeated receptor-mediated endocytosis of intact, bacterially derived minicells as presently recited.

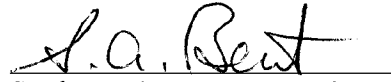
**CONCLUSION**

Applicant submits that the application is in condition for allowance, and an indication to this effect is requested. Examiner Singh is invited to contact the undersigned directly, should she feel that any issue warrants further consideration.

Respectfully submitted,

Date August 18, 2010

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